- 126 -

## CLAIMS:

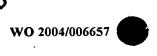
- 1. A method for modulating the levels of a metabolic or biosynthetic product in a plant, including introducing a product into a plant, said method comprising introducing a genetic sequence encoding the product or a precursor to said product, or encoding an enzyme for the biosynthesis or metabolism of the product or a precursor of said product or a genetic sequence which alters the level of expression of a gene encoding a product or an enzyme capable of acting on the product, into a cell or group of cell of said plant, wherein the plant is a member of the family Gramineae family.
- 2. The method of claim 1 wherein the plant is a C4 grass.
- 3. The method of claim 2 wherein the C4 grass is a member of the Saccharum genus.
- 4. The method of claim 3 wherein the member of the Saccharum genus is sugarcane (Saccharum sp. hybrid).
- 5. The method of any one of claims 1 to 4 wherein the product is a polyhydroxyalkanoate.
- 6. The method of claim 4 wherein the polyhdroxyalkanoate is polyhydroxybutryate.
- 7. The method of claim 5 wherein the genetic sequence comprises one or more genetic sequences selected from the list comprising:-
  - (i) a nucleotide sequence encoding a phaA or homolog thereof;
  - (ii) a nucleotide sequence encoding phaB or homolog thereof;
  - (iii) a nucleotide sequence encoding phaC or homolog thereof;

(iv) a nucleotide sequence encoding phaC1 or homolog thereof;

EPLE SA CHANT

WO 2004/006657

- (v) a nucleotide sequence encoding phaG or homolog thereof;
- (vi) a nucleotide sequence encoding phaJ or homolog thereof
- (vii) SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:10 or SEQ ID NO:12 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:1 or SEQ ID NO:3 or SEQ ID NO:10 or SEQ ID NO:12 or a complementary form thereof under low stringency conditions;
- (viii) SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:13 or SEQ ID NO:15 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:4 or SEQ ID NO:6 or SEQ ID NO:13 or SEQ ID NO:15 or a complementary form thereof under low stringency conditions;
- (ix) SEQ ID NO:7 or SEQ ID NO:9 or SEQ ID NO:16 or SEQ ID NO:18 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:7 or SEQ ID NO:9 or SEQ ID NO:16 or SEQ ID NO:18 or a complementary form thereof under low stringency conditions;
- (x) SEQ ID NO:19 or SEQ ID NO:21 or SEQ ID NO:22 or SEQ ID NO:24 or SEQ ID NO:25 or SEQ ID NO:27 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:19 or SEQ ID NO:21 or SEQ ID NO:22 or SEQ ID NO:24 or SEQ ID NO:25 or SEQ ID NO:27 or a complementary form thereof under low stringency conditions;



- (xi) SEQ ID NO:28 or SEQ ID NO:30 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:28 or SEQ ID NO:30 or a complementary form thereof under low stringency conditions;
- (xii) SEQ ID NO:31 or SEQ ID NO:33 or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to SEQ ID NO:31 or SEQ ID NO:33 or a complementary form thereof under low stringency conditions.
- 8. The method of any one of claims 1 to 4 wherein the product is pHBA.
- 9. The method of claim 8 wherein the genetic sequence comprises one or more genetic sequences selected from the list comprising:
  - (i) a nucleotide sequence encoding hydroxycinnamoyl-CoA hydratase/lyase or homolog thereof;
  - (ii) a nucleotide sequence encoding chorismate pyruvate lyase or homolog thereof;
  - (iii) a nucleotide sequence comprising the *ubiC* gene from *E. coli*, or a homolg thereof; and/or
  - (iv) a nucleotide sequence comprising the HCHL gene from Pseudomonas fluorescens or homolg thereof;
- 10. The method of any one of claims 1 to 4 wherein the product is Vanillin.



- 11. The method of claim 10 wherein the genetic sequence comprises one or more genetic sequences selected from the list comprising:-
  - (i) a nucleotide sequence encoding a 3-dehydroshikimate dehydratase and/or;
  - (ii) a nucleotide sequence encoding catechol-o-methyltransferase;
  - (iii) a nucleotide sequence encoding aryl aldehyde dehydrogenase;
  - (iv) a nucleotide sequence encoding feruloyl-CoA synthetase or homolog thereof;
  - (v) a nucleotide sequence encoding enoyl-CoA hydratase or homolog thereof;
  - (vi) a nucleotide sequence encoding enoyl-CoA aldolase or homolog thereof.
- 12. The method of any one of claims 1 to 4 wherein the product is sorbitol.
- 13. The method of claim 12 wherein the genetic sequence comprises one or more genetic sequences encoding a glucose-fructose oxidoreductase or homolog thereof.
- 14. The method of claim 13 wherein the glucose-fructose oxidoreductases is encoded by the polynucleotide sequence set forth in GenBank Accession number Z80356, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to GenBank Accession number Z80356 or a complementary form thereof under low stringency conditions.
- 15. The method of claim 13 wherein the glucose-fructose oxidoreductase is encoded by the polynucleotide sequence set forth in GenBank Accession number M97379, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of



hybridizing to GenBank Accession number M97379 or a complementary form thereof under low stringency conditions.

- 130 -

- The method of any one of claims 1 to 4 wherein the product is indigo. 16.
- The method of claim 16 wherein the genetic sequence comprises one or more 17. polynucleotides selected from the list comprising:
  - a nucleotide sequence encoding L-tryptophan indole lyase or homolog (i) thereof;
  - a nucleotide sequence encoding napthalene dioxygenase or homolog (ii) thereof;
  - a nucleotide sequence comprising the Ralstonia eutropha bec gene or (iii) homolog thereof;
  - the nucleotide sequence set forth in Genbank accession number D14279, or (iv) a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to Genbank D14279 under low stringency conditions.
  - the nucleotide sequence set forth in Genbank accession number M83949, or (v) a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to Genbank M83949 under low stringency conditions.
  - the nucleotide sequence set forth in Genbank accession number AF306552, (vi). or a nucleotide sequence having at least 60% identity thereto after optimal alignment, or capable of hybridizing to Genbank AF306552 under low stringency conditions



- 18. The method of any one of claims 1 to 4 wherein the product is one or more fructan polymers.
- 19. The method of claim 18 wherein the genetic sequence compises one or more genetic sequences selected from the list comprising:-
  - (i) a nucleotide sequence encoding a fructosyltransferase or homolog thereof;
  - (ii) a nucleotide sequence encoding a levan sucrase or homolog thereof.
- 20. The method of claim 19 wherein the fructosytransferase is encoded by the polynucleotide sequence set forth in GenBank Accession number AY150365, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to GenBank Accession number AY150365 or a complementary form thereof under low stringency conditions.
- 21. The method of any one of claims 1 to 4 wherein the product is lactic acid.
- 22. The method of claim 21 wherein the genetic sequence comprises a polynucleotide encoding lactate dehydrogenase.
- 23. The method of any one of claims 1 to 4 wherein the product is adipic acid.
- 24. The method of claim 23 wherein the genetic sequence comprises one or more polynucleotides selected from the list comprising:-
  - (i) a nucleotide sequence encoding a 3-dehydroshikimate dehydratase or homolog thereof;
  - (ii) a nucleotide sequence encoding protochatechuate decarboxylase or



## homolog thereof;

a nucleotide sequence encoding catechol 1,2-dioxygenase or homolog (iii) thereof; and/or

- 132 -

- a nucleotide sequence encoding 3-ketoacyl-ACP synthase or homolog (iv) thereof.
- 25. The method of claim 24 wherein the 3-dehydroshikimate dehydratase is encoded by the aroZ genetic sequence from Klebsiella pneumoniae, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to the aroZ genetic sequence from Klebsiella pneumoniae, or a complementary form thereof under low stringency conditions.
- 26. The method of claim 24 wherein the protochatechuate decarboxylase is encoded by the aroY genetic sequence from Klebsiella pneumoniae, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to the aroY genetic sequence from Klebsiella pneumoniae, or a complementary form thereof under low stringency conditions.
- The method of claim 24 wherein the 1,2-dioxygenase is encoded by the catA 27. genetic sequence from Acinetobacter calcoaceticus, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to the catA genetic sequence from Acinetobacter calcoaceticus, or a complementary form thereof under low stringency conditions.
- The method of claim 24 wherein the 3-ketoacyl-ACP synthase is encoded by the 28. polynucleotide sequence set forth in GenBank Accession Number AF263992, or a homolog thereof having at least 60% identity thereto after optimal alignment, or capable of hybridizing to GenBank Accession Number AF263992, or a complementary form thereof under low stringency conditions.

WO 2004/006657



- 29. The method of any one of claims 1 to 4 wherein the product is 1,3-propanediol.
- 30. The method of claim 29 wherein the genetic sequence comprises one or more polynucleotide sequences selected from the list comprising:-
  - (i) a nucleotide sequence encoding a glycerol dehydratase or homolog thereof;
  - (ii) a nucleotide sequence encoding 1,3-propanediol oxidoreductase or homolog thereof;
  - (iii) a nucleotide sequence encoding glycerol-3-phosphate dehydrogenase or homolog thereof; and/or
  - (iv) a nucleotide sequence encoding glycerol-3-phosphatase or homolog thereof.
- 31. The method of claim 30 wherein the glycerol dehydratase is encoded by the *dhaB* genetic sequence from *Klebsiella pneumoniae*, or a homolog thereof having having at least 60% identity thereto after optimal alignment, or capable of hybridizing to said *dhaB*, or a complementary form thereof, under low stringency conditions.
- 32. The method of claim 30 wherein the 1,3-propanediol oxidoreductase is encoded by the *dhaT* genetic sequence from *Klebsiella pneumoniae*, or a homolog thereof having having at least 60% identity thereto after optimal alignment, or capable of hybridizing to said *dhaT*, or a complementary form thereof, under low stringency conditions.
- 33. The method of any one of claims 1 to 4 wherein the product is 2-phenylethanol.
- 34. The method of claim 33 wherein the genetic sequence comprises one or more polynucleotide sequences selected from the list comprising:-



- 134 -
- a nucleotide sequence encoding a aromatic-L-amino acid decarboxylase or (i) homolog thereof;
- a nucleotide sequence encoding 2-phenylethylamine oxidase or homolog (ii) thereof; and/or
- a nucleotide sequence encoding aryl-alcohol dehydrogenase or homolog (iii) thereof.
- A vector comprising one or more of the genetic sequences recited in any one of 35. claims 1 to 34.
- The vector of claim 35 wherein the vector is an expression vector. 36.
- A genetically modified cell comprising one or more of the genetic sequences 37. recited in any one of claims 1 to 34, or the vector of claim 35 or 36.
- The genetically modified cell of claim 37 wherein the cell is a Saccharum sp. cell. 38.
- A genetically modified plant comprising cells comprising one or more of the 39. genetic sequences recited in any one of claims 1 to 34, or the vector of claim 35 or 36.
- The genetically modified plant of claim 39 wherein the plant is the C4 grass 40. sugarcane.
- Seeds or other reproductive material from the plant of claim 39 or 40. 41.
- A product produced in transgenically modified plant by the method of any one of 42. claims 1 to 34.

- 43. The product of claim 42 wherein the product is a polyhydroxyalkanoate polymer or mixture of polyalkanoate polymers.
- 44. The product of claim 43 wherein the product is polyhydroxybutyrate.
- 45. The product of claim 43 wherein the product is pHBA.
- 46. The product of claim 43 wherein the product is vanillin.
- 47. The product of claim 43 wherein the product is sorbitol.
- 48. The product of claim 43 wherein the product is indigo.
- 49. The product of claim 43 wherein the product is a fructan polymer or mixture of fructan polymers.
- 50. The product of claim 43 wherein the product is lactic acid.
- 51. The product of claim 43 wherein the product is adipic acid.
- 52. The product of claim 43 wherein the product is 1,3-propanediol.
- 53. The product of claim 43 wherein the product is 2-phenylethanol.
- 54. A plant based bioreactor system used for the production of a metabolic or biosynthetic product, said bioreactor comprising one or more sugarcane (Saccharum sp.) plant(s) produced according to the method of any one of claims 1 to 34.